OBSERVATIONS ON THE CYSTS OF THE COMMON INTESTINAL PROTOZOA OF MAN

BY

J. R. MATTHEWS, M.A.

From the Liverpool School of Tropical Medicine

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INTRODUCTION

The following remarks are presented mainly by way of an explanation of the accompanying plate which has been prepared for this journal at the request of the Editors. Considering the large amount of descriptive work already published on the human intestinal protozoa, it is not to be anticipated that much that is new will here be added to our knowledge of the morphology of the cysts of these organisms. Yet it was considered desirable that the present opportunity, when so much material is available, should not be allowed to pass without making some drawings of the cysts commonly found during the routine examination of the stools of convalescent dysenterics. It is recognised that these cysts have been figured many times by many different authors, but the figures have generally been drawn from fixed and stained preparations, and while some are excellent it must be admitted that others are less so. On the other hand, figures have appeared which are obviously diagrammatic. The need was felt for a series of drawings depicting the common human protozoal cysts as they may be seen by the student of Tropical Medicine or by anyone engaged on routine work for the purpose of diagnosis. With this object in view the present figures have been prepared. To one who has had considerable experience, it is often sufficient to examine the cyst-containing material in saline, but if the material be mounted in iodine solution,* the cysts become stained, and the nuclei, which were perhaps invisible or difficult to see, will be found, as a rule, to stand out more clearly defined. The majority

* Weigert's solution = iodine 1, potassium iodide 2, distilled water 100.

of the figures which appear on the plate were made from cysts stained in this way. While it is admitted that exact cytological detail cannot be obtained by this method, it is nevertheless true that the essential features of most cysts can be ascertained, and it is the method generally adopted in practice when a diagnosis has to be made. It is worth bearing in mind, however, that no matter how well defined a cyst may appear in iodine, it is not easy to represent the appearance in a black and white drawing, which lacks the coloration which the iodine gives—a feature, in my opinion, of considerable importance.

Notes on the appearance of cysts stained with iodine will be given under each protozoon described.

ENTAMOEBA HISTOLYTICA

One of the most important of recent discoveries regarding this organism is the fact that there is great variation in the size of its cysts. It is now known that infections occasionally occur in which the cysts are well over 15 microns in diameter, and it is definitely established that in a large number of infections the cysts are well below 10 microns. Thus, instead of stating that *E. histolytica* produces cysts varying from 10μ to 15μ in diameter, it is now more correct to state that the size ranges from 5μ to 20μ. It is not to be supposed, however, that cysts having this wide range in size will be found in every infected case. Such an occurrence is exceptional. An analysis of the results obtained in this laboratory during the past year shows that of the total number of *E. histolytica* infections detected about 35 per cent. consisted of cysts varying from 6μ to 9μ, while in the remaining infections, the great majority of the cysts varied from 10μ to 15μ in diameter. A few individual cysts about 18μ have been observed, and a few cases were found where the infection obviously consisted of both large and small cysts, and all gradations in size from 6μ to 15μ were observed. The small cysts have been noted by various workers, and in an important paper Wenyon and O'Connor (1917) have given a careful study of them, and have come to the conclusion that they belong to a distinct race or strain of the species *E. histolytica*. This view is also held by Dobell and Jepps who, in a recent and valuable paper (1918), have

demonstrated the existence of several races within the species, distinguishable from one another by the size of the cysts which they produce. In the accompanying plate small cysts of *E. histolytica* are shown in figs. 1-4, and these should be compared with figs. 5-9, which depict larger cysts of the same species.

The importance of the foregoing remarks on the size of *E. histolytica* cysts is at once obvious, when it is remembered that in human faeces other cysts occur, of similar sizes and of somewhat similar appearance, with which the cysts of *E. histolytica* may possibly be confused. On the one hand, the cysts of *E. nana* which are 7μ or 8μ in diameter may be mistaken for the small cysts of *E. histolytica*, and on the other, small cysts of *E.coli* at certain stages in their development may be confused with the larger cysts of *E. histolytica*. We are thus compelled to abandon size of cyst as a certain criterion for diagnostic purposes. Of greater importance, as has been often pointed out, and again emphasised by Dobell and Jepps (1917), is the structure and number of the nuclei. The fully developed cyst of *E. coli* has eight while the fully developed cyst of *E. histolytica* has four nuclei. We shall deal with this tetranucleate stage first. If it is a small cyst of 7μ or 8μ in diameter it can be distinguished from a tetranucleate cyst of *E. nana* by the structure of its nuclei (compare figs. 4 and 10). Further, the nuclei of an *E. histolytica* cyst are nearly always clearly defined in iodine solution, while in the vast majority of *E. nana* cysts it is extremely difficult to distinguish the nuclei clearly (figs. 12 and 13). If the cyst is large, and comes within the range of the size of *E. coli* cysts, we are almost entirely dependent on the structure of the nuclei for a correct diagnosis (compare figs. 8 and 14). Even if the two cysts depicted in these figures had been of the same size, the nuclear structure would still provide a differential character. In my experience, however, the tetranucleate stage of *E. coli* is not commonly found, though this, of course, does not dispose of the difficulty. The appearance of the cytoplasm is often of considerable help, for it generally stains less uniformly in *E. histolytica* than in *E. coli* cysts. If chromatoid bodies (chromidial bars) are present, their shape is of some value. In *E. histolytica* they are commonly square or round-ended rods, while sharp-pointed, ragged-ended, or large irregular blocks are more characteristic of *E. coli*.

We may now briefly refer to *E. histolytica* and *E. coli* cysts containing fewer than four nuclei. The uninucleate and binucleate stages of *E. histolytica* are quite common. They are less common in *E. coli*. There should, however, be little difficulty in distinguishing the two species in these stages of development. The difference in the structure of the nuclei is well known (see figs. 5, 6, 7, 15 and 16). In these stages, also, vacuoles which contain glycogen are common in both species, and the appearance of these in iodine is important. In *E. histolytica* the vacuole is generally ill-defined and not very deeply stained (figs. 1, 5 and 6). In binucleate cysts of *E. coli* the vacuole is commonly large, occupying nearly the whole cyst, and is always deeply stained with well defined edges (fig. 15). In some binucleate *E. coli* cysts, however, the vacuole appears crescent-shaped, round one side of the cyst. Not uncommonly *E. histolytica* cysts possess numerous vacuoles (a type which is frequently encountered in relapses after treatment) and these do not always stain in iodine (fig. 7). On the other hand, uninucleate and binucleate cysts of both species occur not infrequently without vacuoles, in which case the nuclear structure remains as a differential diagnostic character.

ENTAMOEBA COLI

The difficulties that are likely to be encountered in distinguishing this species in certain stages of encystment have already been noted. The fully developed cyst containing eight nuclei is the stage most commonly found, and cannot possibly be mistaken if the nuclei are carefully counted, even when its dimensions are within the range of size of *E. histolytica* cysts (see fig. 17 and compare with fig. 8). *E. coli* cysts show great variation in size, spherical cysts ranging from about 12μ to over 30μ in diameter. Dobell and Jepps have measured *E. coli* cysts as small as 11μ, and Wenyon and O'Connor have recorded 38μ by 34μ as maximum values. These larger cysts contain as a rule sixteen nuclei. Chromatoid bodies are not uncommon in *E. coli* cysts, and the old distinction of their presence in *E. histolytica* and their absence in *E. coli* cannot be any longer maintained. As already noted, these bodies, which are best studied in cysts mounted in saline, are as a rule less regular in shape in

E. coli than in *E. histolytica* (compare figs. 2, 9 and 18). A fairly common appearance of the eight-nucleate *E. coli* cyst is shown in fig. 19. There is a central mass of granular cytoplasm, in which several or all of the nuclei are embedded, surrounded by more alveolar and less deeply staining cytoplasm. The nuclei are often more or less disintegrated or distorted in appearance, and the condition is probably abnormal, although James (1914) regards the phenomenon as a normal stage in encystment.

In a paper on the measurements of cysts which appears in this number of the *Annals of Tropical Medicine and Parasitology*, Smith (1918) has dealt fully with the differences between the cysts of *E. histolytica* and *E. coli*, and has drawn up a diagnostic table giving the main points of distinction. It may be useful to give a similar tabular statement in the present paper, but it should be borne in mind that while the distinctions apply generally, some of them may break down in individual instances.

E. histolytica cysts.	*E. coli* cysts.
Size, 5μ to 20μ in diameter, most commonly 6μ to 15μ.	Size, 11μ to 35μ in diameter, most commonly 14μ to 22μ.
Nuclei 1 to 4 in number.	Nuclei 1 to 8, occasionally 16.
Peripheral chromatin of nucleus of small granules more or less evenly distributed.	Peripheral chromatin of nucleus of larger unevenly distributed blocks.
Cytoplasm greenish in fresh condition, typically not uniform in appearance.	Cytoplasm greyish in fresh condition, more uniform in appearance.
Vacuoles one or more, usually faintly stained by iodine with ill-defined edges.	Vacuole generally single, usually deeply stained by iodine with sharply defined edges.
Chromatoid bodies more frequent, rod-shaped with square or rounded ends.	Chromatoid bodies less frequent, irregular in shape with pointed or splintered ends.

ENTAMOEBA NANA

This entamoeba, named by Wenyon and O'Connor (1917), has been fully described by these authors and by Dobell and Jepps (1917). Its cysts are frequently encountered in the examination of both dysenteric and normal stools, and as already mentioned, it is important to distinguish them from the small cysts of *E. histolytica*. They are oval or roundish structures about 8μ to 10μ long, or 7μ or 8μ in diameter. They contain numerous highly refringent

granules, and if they are allowed to stain in iodine for some time (fifteen minutes or longer) it is often possible, in some of the cysts at least, to make out the nuclei, which vary in number from one to four according to the stage of development. The tetranucleate cyst shown in fig. 10 was uncommonly well defined, and figs. 12 and 13 depict what is more generally seen. The exact structure of the nucleus, with its large, usually eccentric karyosome, can only be obtained by staining with iron haematoxylin or some other suitable stain. A feature of *E. nana* cysts which is common in the binucleate stage, though not so frequent in the tetranucleate, is the presence of a large glycogen-containing vacuole which stains dark brown in iodine (fig. 11). It is, moreover, clearly defined, and this fact should be of service in distinguishing these cysts from the small cysts of *E. histolytica*, in which the vacuole is less deeply stained and not so sharply defined.

CHILOMASTIX (TETRAMITUS) MESNILI

The cysts of this flagellate may possibly be confused with those of *Entamoeba nana* (compare figs. 11 and 23). They are about the same size, but the characteristic protuberance at one end of a Tetramitus cyst, giving it a lemon-shaped appearance, is a feature which should help in the determination of the great majority of the cysts of the flagellate. Oval and spherical cysts have been described by Dobell and Jepps, but they do not appear to be common. The nuclear structure is again one of the most important characters, and its essential features can readily be observed in cysts that have been allowed to stain in iodine for ten minutes or longer. The nucleus is large, and possesses at one pole a distinct mass of chromatin giving the appearance of a signet ring (fig. 22). In addition, staining with iodine brings out quite clearly the outline of the remains of the cytostome of the flagellate, and this feature should simplify the identification of the cyst. Occasionally the cysts of *T. mesnili* contain a large, well defined mass of glycogen which stains dark brown in iodine (fig. 23). It often renders the cytostome invisible, and when this is the case care should be taken not to confuse the cyst with that of *E. nana* or with small forms of iodine cysts, which will be referred to below.

GIARDIA (LAMBLIA) INTESTINALIS

The cysts of this flagellate are among the most easily recognised of all the protozoal cysts occurring in human faeces. They are nearly always oval-shaped structures, though occasionally almost spherical forms have been observed, and these may bear a fairly close resemblance to cysts of *E. histolytica*. Mounted in iodine the internal structure of the cyst can readily be distinguished. Most commonly four nuclei are present, generally lying towards one end of the cyst, and remains of the intracytoplasmic structure of the flagellate are extremely characteristic (fig. 20). Staining with iodine brings out a striking difference between two kinds of Lamblia cysts which has not hitherto been noted. In almost every infection, if examined sufficiently carefully, it will be found that some of the cysts stain brown while others are stained a bluish-grey or slate colour. Observations show that in conjunction with the different staining reaction there is a distinct difference in the size of the two kinds of cysts. The brown ones are larger, the majority varying from 12μ to 15μ in length, while the blue staining cysts are smaller, the average length being 10μ or 11μ (figs. 20 and 21). As already mentioned, the differentiation between these two forms of cyst is most marked when they are examined in iodine, because of their different colour, but they can also be distinguished in saline partly by the difference in size and partly by the more granular and somewhat degenerate appearance of the smaller and bluish-staining type. What these differences signify is not yet determined.

IODINE CYSTS

These cysts were first noted by Wenyon (1915), and have been more fully described by Wenyon and O'Connor (1917). They vary greatly in size, covering practically the same range in size as the cysts of *E. histolytica*, and it is with these that they are most likely to be confused. This is particularly the case when the structures are examined in saline. The iodophilic body which is most characteristic of Iodine Cysts may simulate the chromatoid body of an *E. histolytica* cyst, though it is generally not rod-shaped but rounded or lobed. In iodine this inclusion stains deep brown and

has well-defined edges. Occasionally, Iodine Cysts are found without the iodophilic body, and it is then that the organism may very closely resemble a cyst of *E. histolytica*. The nucleus is, however, distinctly smaller than the nucleus of a uninucleate *E. histolytica* cyst, and is quite different in structure (see fig. 24 and compare with figs. 1, 2, and 5). (Fig. 24 was drawn from fixed material stained with iron haematoxylin. The iodophilic body shows as a clear space, but in a cyst mounted in iodine this space would appear dark brown in colour and the nuclear structure would be less clearly defined). It should be noted also that small Iodine Cysts may be confused with *E. nana* or Tetramitus cysts when these contain, as they sometimes do, deeply staining glycogen vacuoles. Iodine Cysts are not protozoal. They probably represent some stage in the life-history of a vegetable organism but so far all attempts to cultivate them have failed.

BLASTOCYSTIS HOMINIS

This organism, like that described under the name of Iodine Cyst, is also of a vegetable nature. It is exceedingly common in human faeces, and may possibly be mistaken for some of the protozoal cysts already dealt with, and in particular, it may be confused with encysted stages of *E. histolytica*. Blastocystis varies in size from 5μ to over 20μ in diameter. A large number of infections consist only of small forms, about 7 or 8 microns, and these may occasionally be troublesome to distinguish from small cysts of *E. histolytica*. Blastocystis, possesses, however, a much thinner enveloping membrane, within which there is a narrow rim of cytoplasm, nearly always most conspicuously developed at opposite poles of the structure. The centre of the organism is occupied by a reserve body, but in many instances this body has disappeared and the structure then apparently possesses a central vacuole (fig. 25). In the cytoplasm a varying number of nuclei occur. Considerable variety of form is met with in Blastocystis. Dumb-bell shaped individuals are not infrequently encountered, representing a stage in the transverse division of the organism, and multiple reproductive cysts, which are considerably larger in size than single forms, have been described by Alexeieff and Wenyon and O'Connor.

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EXPLANATION OF PLATE

All the figures (except 9 and 24) were drawn, from cysts stained with iodine, with the aid of a camera lucida at a magnification of 3,300. They have been reduced in reproduction.

Figs. 1-9. Cysts of *Entamoeba histolytica.*

Fig. 1. Uninucleate cyst containing vacuole. Diameter 8μ
Fig. 2. Uninucleate cyst with a single chromatoid body. Diameter 8μ
Fig. 3. Binucleate cyst. Diameter 7·5μ
Fig. 4. Tetranucleate cyst. Diameter 8μ
Fig. 5. Uninucleate cyst with diffuse staining vacuole, size 13·5μ × 12μ
Fig. 6. Binucleate cyst with diffuse staining vacuole. Diameter 12μ
Fig. 7. Binucleate cyst with several vacuoles unstained by iodine. Diameter 10μ
Fig. 8. Tetranucleate cyst. Diameter 12·5μ
Fig. 9. Binucleate cyst with typical chromatoid body. Diameter 11μ. Cyst as seen in saline.

Figs. 10-13. Cysts of *Entamoeba nana.*

Fig. 10. Tetranucleate cyst, nuclei remarkably clear. Size 9μ × 8μ
Fig. 11. Binucleate cyst with deeply stained mass of glycogen. Size 9μ × 7·5μ
Fig. 12. Binucleate cyst, nuclei faintly stained. Size 8·5μ × 7μ
Fig. 13. Uninucleate cyst, nucleus faintly stained. Size 8·5μ× 7μ

In all four cysts the refringent granules of volutin were clearly discernible.

Figs. 14-19. Cysts of *Entamoeba coli.*

Fig. 14. Tetranucleate cyst. Diameter 17·5μ
Fig. 15. Binucleate cyst with large deeply stained centrally placed vacuole. Diameter 16μ
Fig. 16. Binucleate cyst without vacuole. Diameter 18·5μ
Fig. 17. Eight nucleate cyst. Diameter 13μ
Fig. 18. Eight nucleate cyst with pointed chromatoid bodies. Diameter 16μ
Fig. 19. Probably abnormal eight nucleate cyst. Diameter 17μ

Figs. 20-21. Cysts of *Giardia intestinalis.*

Fig. 20. Large brown staining cyst. Size 15μ × 9·5μ
Fig. 21. Small bluish-grey staining cyst. Size 10μ × 6·5μ

Figs. 22-23. Cysts of *Chilomastix mesnili.*

Fig. 22. The cytostome and nucleus clearly visible. Size 8·5μ × 7·5μ
Fig. 23. The cytostome and nucleus partially obscured by large deeply staining mass of glycogen. Size 8·5μ × 7·5μ

Fig 24. Iodine cyst drawn from specimen stained with iron haematoxylin. The clear space would appear dark brown in colour if stained with iodine and the nucleus would be less sharply defined. Diameter 11·5μ

Fig. 25 *Blastocystis hominis.* Diameter 10μ